

Original Article

Ultrastructure and Cytoarchitecture of Bachmann's Bundle in the Mammalian Heart

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Recently, the Bachmann's bundle (BB) has been examined in connection with atrial fibrillation. However, the morphological properties of the BB remain to be clarified.

In this study, the BB in hearts of monkeys and sheep was investigated by immunohistochemistry, scanning (SEM) and transmission (TEM) electron microscopy.

Immunohistochemically, BB myocytes showed a strong positive reaction for desmin antibody and that connexin (Cx) 40 and Cx43 were distributed at the intercalated disks. BB myocytes were characterized by a dense network of intermediate filaments which enveloped nucleus, myofibrils and mitochondria, respectively. The intercalated disks showed an irregular stair-like profile. Microprojections on the steps were smaller in number and larger in size than those in auricular myocytes.

In conclusion, BB myocytes were different ultrastructurally from auricular myocytes, showing morphological properties of the conduction system.
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Key words: Bachmann's bundle, intercalated disk, intermediate filament, Cx40, morphology

Introduction

In 1916, G. Bachmann reported on the auricular time intervals in the American Journal of Physiology, and hypothesized that a specialized muscular bundle may function as interauricular conduction.¹⁾ Thereafter, this muscular bundle has come to be called as the Bachmann's bundle (BB). Further, James (1963)^{2,3)} reported that the internodal pathway, connecting between the sinoatrial (SA) node and the atrioventricular node (AV node, Tawara's

node), appears to consist of anterior, middle and posterior internodal pathways. The anterior internodal pathway was divided into two muscular bundles, and the BB was one of them. The BB is currently understood to be a typical interatrial pathway. So far many findings in the cytoarchitecture of the working cardiac myocytes and the myocytes in the cardiac conduction system have been clarified.^{4–9)} However, it has not yet been demonstrated whether or not myocytes in the internodal pathway including the BB are different in structure from atrial working

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myocytes. Compared with the working cardiac myocytes, the myocytes of the conduction system generally contained smaller and fewer mitochondria, approximately in proportion to the degree of development of myofibrils.^{10,11)} Although atrial special granules as a secretory function were not generally found in the conduction tissue,^{12,13)} myocytes in the human internodal tract exhibited an intensity comparable to that of atrial natriuretic peptide (ANP) granules.¹⁴⁾ Furthermore, Purkinje cells in the unguate had a greater number of intermediate filaments than working cardiac myocytes.¹⁵⁾ Intermediate filaments are made from desmin,¹⁶⁾ and Thornell (1985)¹⁷⁾ reported that the desmin in bovine Purkinje cells was distributed throughout the cytoplasm. However, there are no reports on the distribution of intermediate filaments in the specialized cardiac muscles except bovine Purkinje cells.

Cardiac myocytes were connected with each other end-to-end at the intercalated disks. The intercalated disks consisted of desmosomes, fascia adherentes and gap junctions. It is known that the cardiac conduction system, excluding the SA and AV node, contains connexin 40 (Cx40) and connexin 43 (Cx43). The conductance of Cx40 was greater than that of Cx43.^{18,19)} Recently the intercalated disks in the mammalian heart were visualized three-dimensionally by utilizing scanning electron microscopic techniques combined with the NaOH/ultrasonication method.^{20–22)} The intercalated disks of myocytes in the crista terminalis and surrounding the fossa ovalis were apparently different in three-dimensional architecture from those of atrial myocytes.²¹⁾

Thus, in spite of the numerous morphological studies on the working myocardium and the cardiac conduction system, relatively little attention has been paid to the BB for many years.²³⁾

Recently, it has been suggested that Bachmann's bundle pacing is effective for the prevention of atrial fibrillation (AF),^{24,25)} and many clinical and experimental data about AF and BB have also been reported.^{26–33)} The significance of BB pacing appears to have been demonstrated clinically and electrophysiologically. The morphological characteristics of the BB is of great practical important. Therefore, we examine the functional morphology of the BB in the monkey and sheep heart using various anatomical and histological techniques.

Materials and Methods

The hearts of five adult Japanese macaque monkeys and five adult sheep were used in this study. The sheep hearts have been classically used

for morphological study of the cardiac conduction system because the specialized cardiac muscle are easily identified at the macroscopic and microscopic levels. On the other hand, the Japanese macaque is a Primate and is closely akin to humans. All experimental and animal care procedures were approved by the Institutional Animal Care Committee of Oita University. The Japanese macaque monkeys and sheep were deeply anesthetized by intraperitoneal injection of sodium pentobarbital, and hearts were removed. Atria including the BB were fixed with Karnovsky's fixative or 4% paraformaldehyde fixative at 4 °C for a few days. The right auricular appendage (RAA), left auricular appendage (LAA) and BB were dissected, and were investigated by light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). For light microscopy, tissue blocks were fixed in 4% paraformaldehyde and embedded in paraffin, and paraffin blocks were cut at a 7 µm thickness. Sections were immuno-stained with anti-desmin, and Cx40 and Cx43 antibodies, respectively, according to the immunogold-silver method.³⁴⁾ For SEM, tissue blocks of the right auricle and BB were fixed in Karnovsky's fixative, and were treated with 2N NaOH for 2–3 h at room temperature in order to view the cytoskeleton of cardiac muscles. On the other hand, other tissue blocks were treated with 6N NaOH for 10 min at 61 °C and then placed in an ultrasonic bath (38 kHz) for a supplementary 1 min to investigate the cytoarchitecture and intercalated disks. The specimens were dehydrated, dried by the t-butylalcohol drying method, coated with both gold and osmium gas, and observed under a Hitachi S-800 scanning electron microscope. For TEM, small tissue blocks were fixed in Karnovsky fixative and 2% osmium tetroxide and embedded in Epon. Ultra-thin sections were stained with uranyl acetate and lead citrate, and viewed under a JEOL-100CX transmission electron microscope.

Results

1. Macroscopic observation

When the thin transverse sinus of the pericardium in the monkey and sheep hearts was opened and the posterior surface of the atria was disclosed, a muscular bundle, the so-called Bachmann's bundle (BB), connecting between the right atrium and the left atrium, was identified macroscopically (**Figure 1**). Muscle fibers of the BB appeared to run in a straight line (**Figure 1**). The BB of the sheep heart was larger and wider than that of the monkey heart (Data omitted).

Figure 1 Macroscopic image of the posterior surface of the sheep atrium. The Bachmann's bundle (BB) connects between the right and left auricle. Arrow indicates the BB.
SCV: superior caval vein, LPV: left pulmonary vein, RAA: right auricular appendage, LAA: left auricular appendage

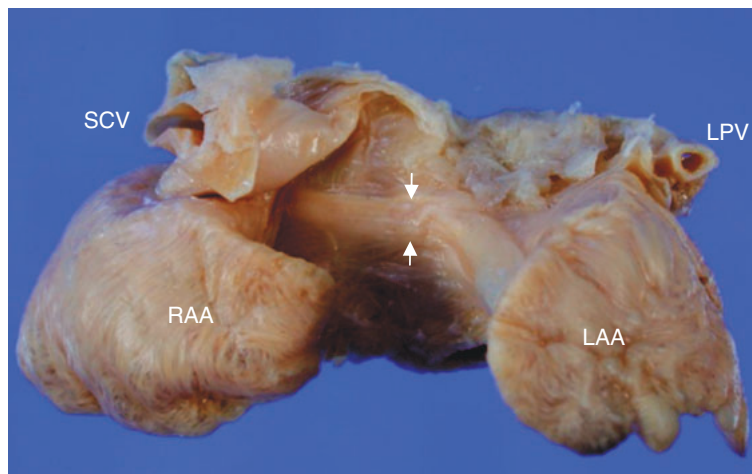
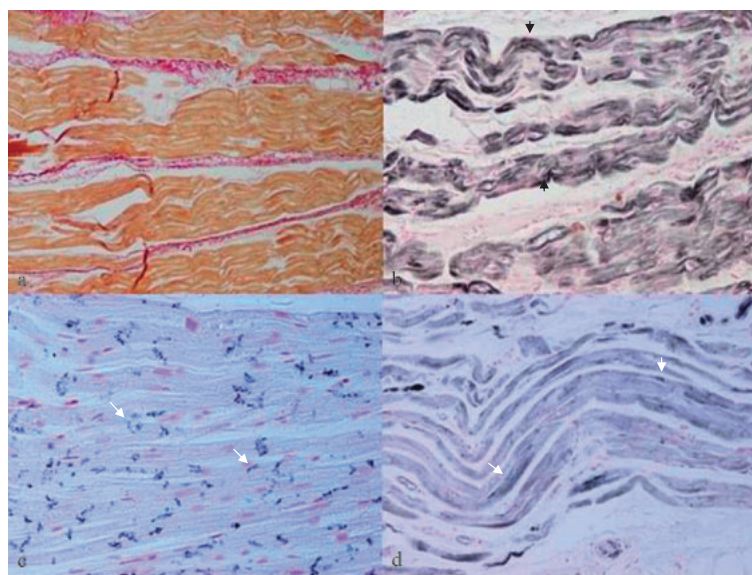


Figure 2 Light micrographs of the monkey BB.
a) Myocytes in the BB appear to run in a straight line. b) Myocytes in the BB show a positive reaction for desmin antibody (arrow). c) The intercalated disks in the BB show a positive reaction for connexin 40 (Cx40) antibody (arrow). d) They also have connexin 43 (Cx43) (arrow). (a~d: $\times 400$)



2. Light microscopic findings

Cardiac myocytes of the BB in the monkey and sheep hearts were arranged in a straight line and gave a lighter appearance due to the smaller number of myofibrils (**Figure 2a**). In immuno-stained specimens, cardiac myocytes of the monkey BB showed a stronger positive reaction for desmin antibody (**Figure 2b**) as compared with auricular myocytes. The reactions appeared to spread widely throughout the cytoplasm. In addition, cardiac myocytes of the BB were connected end-to-end at the intercalated disks, and sometimes had side-to-side junctions. The intercalated disks had both Cx40 and Cx43 which indicated fast conductance (**Figures 2c,d**). Of special note, a Cx40 positive reaction was matched to the intercalated disks (**Figure 2c**).

3. Ultrastructure of working cardiac myocyte (auricular myocytes)

In TEM and SEM images, the atrial working myocytes in the monkey and sheep heart were characterized by the presence of abundant myofibrils and a lot of mitochondria with a large size (about $1.5\mu\text{m}$ in a long diameter) (**Figures 3a,b**). Mitochondria were distributed at two poles of the nucleus between myofibrils and beneath the sarcolemma (**Figure 3a**). A few intermediate filaments of 10 nm in diameter were distributed near the intercalated disks. Working cardiac myocytes in the right and left auricle had a lot of atrial natriuretic poripeptide (ANP) granules in the cytoplasm, especially around the nucleus (**Figure 3a**). They were also seen between myofibrils and near the cell membrane.

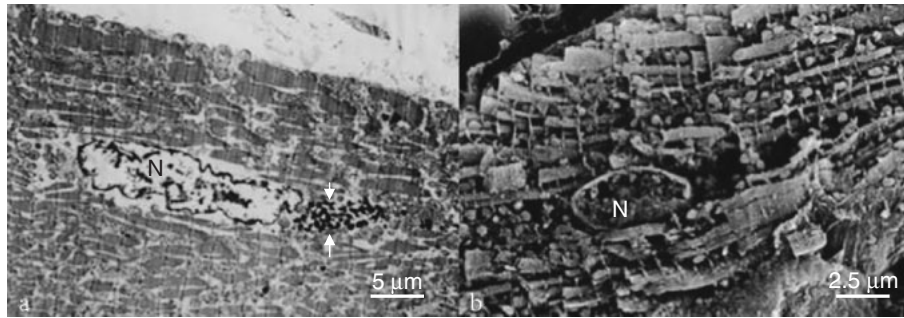


Figure 3 TEM (a) and SEM (b) images of auricular myocytes. The myocytes have abundant myofibrils and large mitochondria. Near the nucleus (N), a lot of atrial specific granules (arrow) can be seen. (a: $\times 2,000$, b: $\times 4,400$)

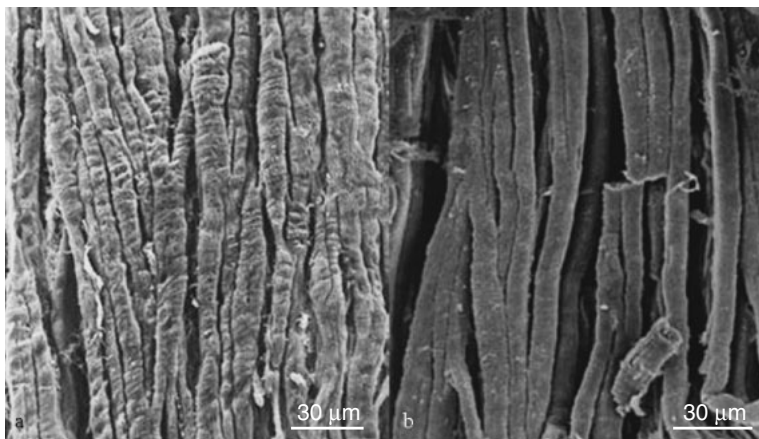


Figure 4 SEM images of the auricle and the BB.

a) Auricular myocytes are connected with each other at the ends of branches and form a complicated three-dimensional network. **b)** Myocytes in the BB appear to run in a straight line. (a: $\times 770$, b: $\times 840$)

TEM observations demonstrated that the intercalated disks of working cardiac myocytes consisted of transversely arranged plicate segments and longitudinally arranged interplicate segments, as demonstrated in another paper.²⁰⁾ The plicate segments showed a long zigzag profile and had many desmosome and small gap junctions. The interplicate segments had large gap junctions. On the other hand, the NaOH/ultrasonication treatment of cardiac tissues enabled us to view not only the cytoarchitecture but also the intercalated disks three-dimensionally (Figures 3b, 8a). Atrial working myocytes in the sheep and monkey heart were fundamentally cylindrical in shape, often gave rise to a few branches, and were connected with each other at the ends of branches to form a complicated network (Figure 4a). The intercalated disks showed a short stair-like profile. They consisted of steps and risers. The former corresponded to the plicate segments and had many microprojections and microridges. The latter corresponded to the interplicate segments.

4. Ultrastructure of Bachmann's bundle

In TEM images, cardiac myocytes of the BB in the

monkey hearts were characterized by the presence of small amounts of myofibrils and small mitochondria, and had a large space around the nucleus (Figure 5a). Some ANP granules existed around the nucleus, between myofibrils and near the sarcolemma (Figure 5b). Mitochondria were about $0.75\ \mu\text{m}$ in long diameter, being smaller in number than those of auricular myocytes. Higher magnification views illustrated clearly that numerous intermediate filaments of $10\ \text{nm}$ in diameter were distributed around the nucleus and between myofibrils (Figures 5b, 6). In SEM images of the BB treated with 2N NaOH, myofibrils, mitochondria and intermediate filaments were observable three-dimensionally (Figure 6). Myofibrils were abundantly located at the periphery of the cells. Mitochondria were generally spherical in shape and smaller in size than those in auricular myocytes. Intermediate filaments showed a woolen thread-like appearance. Especially, they were abundantly distributed not only around nuclei but also between myofibrils. The filaments also surrounded mitochondria.

The intercalated disks in myocytes of the BB did not form a regular zigzag formation, as shown in the

Figure 5 TEM images of the monkey BB. **a)** Myofibrils were entirely distributed at the periphery of the cells, and mitochondria with an oval shape are scattered here and there. **b)** A few atrial specific granules (arrow) are present near the nucleus. N, nucleus; M, mitochondria. (a: $\times 1,700$, b: $\times 14,700$)

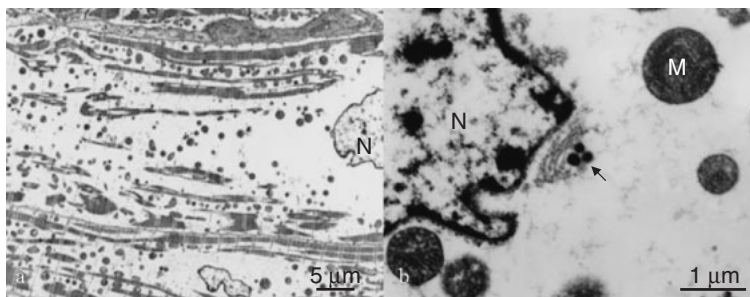


Figure 6 SEM images of the monkey BB treated with 2N NaOH. Intermediate filaments with a dense network are distributed near nucleus (N), between myofibrils and around mitochondria. Inset shows intermediate filaments (arrow) surrounding the nucleus (N) and mitochondria (M). ($\times 7,000$, inset: $\times 14,000$)



plicate segments of auricular myocytes and showed an ambiguous zigzag. Desmosomes were small in number (**Figure 7a**). The interplicate segments with large gap junctions ran obliquely (**Figures 7a,b**). In SEM images of the BB treated with 6N NaOH followed by ultrasonication, cardiac myocytes were cylindrical in shape and sometimes bifurcated. They ran in fairly straight lines (**Figure 4b**). Although the myocytes in the BB relatively resembled the cytoarchitecture of auricular myocytes, they differed considerably from auricular myocytes with respect to the three-dimensional architecture of the intercalated disks. The intercalated disks in the BB showed an

irregular stair-like profile and contained three or four steps and risers (**Figure 8b**). Microprojections on the steps were from 0.15 to 0.54 μm in size. They were larger in size and smaller in number than those in auricular myocytes. Microprojections in auricular myocytes were from 0.10 to 0.21 μm in size.

Discussion

At first, no essential difference in morphology of the BB between the sheep and the monkey was observed. It is known that the BB is the muscular bundle connecting the RAA and LAA and is also one

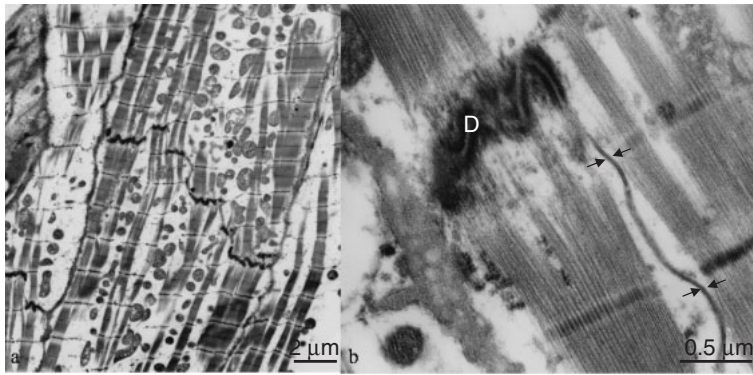


Figure 7 TEM images of the intercalated disks in the BB.

a) They show an ambiguous zigzag, and desmosomes are small in number. **b)** Large gap junctions (arrows) were present in the interplate segments. (a: $\times 3,700$, b: $\times 34,200$) D: desmosome.

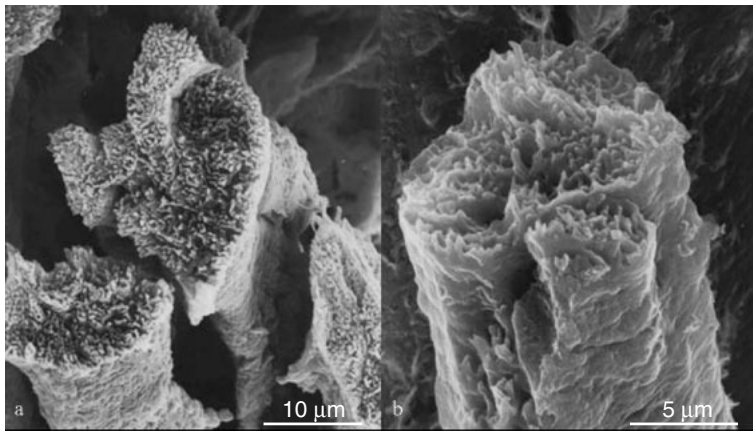


Figure 8 SEM images of the auricle and the BB treated with 6N NaOH/ultrasonication.

The intercalated disks can be seen three-dimensionally. **a)** The intercalated disks in auricular myocytes show a regular stair-like profile, and there are a great number of microprojections on the steps. **b)** Those in the BB show an irregular stair-like profile, and microprojections are obviously small in number. (a: $\times 4,800$, b: $\times 8,000$)

of the internodal pathway. Ultrastructure of myocytes in the BB remained to be unclear. Certain interesting structural differences are noted between the atrial working myocardium (auricular muscle) and the BB. The atrial and ventricular working myocardium showed the following ultrastructural characteristics to carry out a pump function. The cytoplasm was occupied by numerous myofibrils and large mitochondria with well-developed cristae. The mitochondria were abundantly distributed around the nucleus, between the myofibrils and beneath the sarcolemma.^{4,11)} The present TEM and SEM studies illustrated that myocytes in the BB were less dense in appearance and had peripherally-distributed myofibrils and mitochondria with a small size. Mitochondria were scattered here and there. The present SEM and TEM studies also demonstrated that myocytes of the BB in the sheep and monkey heart connected end-to-end at the intercalated disks and ran in straight lines. This straight course seems to be advantageous for transmission excitation. As speculated by Bachmann, the BB need not only synchronized contraction but also fast conductance in order to transmit excitation from the right atrium

to the left atrium. Furthermore the present immunohistochemical study illustrated that the BB had both Cx40 and Cx43. It has also established that Cx40 is expressed in the cardiac conduction system, except for the SA and AV node, and possessed fast conductance.^{18,19)} These morphological findings may indicate that myocytes in the BB belonged to the fast conduction type.

In addition, it should be noted that myocytes in the BB showed a strong reaction for desmin antibody. Desmin (originally named skeletin) belongs to the family of intermediate filaments.¹⁶⁾ In general, it has been well known that the function of intermediate filaments is to maintain muscle cytoarchitecture. A few intermediate filaments in the working myocardium appeared to exist between myofibrils,^{5,11)} while those in the BB formed a dense network and were distributed near the nucleus, around mitochondria and between myofibrils. These findings in the BB resembled Purkinje fibers in the ungulate heart.¹⁷⁾ It is known in the normal situation that Purkinje fibers have especially more intermediate filaments than working cardiac myocytes.¹¹⁾ It is concluded that intermediate filaments in BB myocytes take an

important role in the cytoarchitecture. On the other hand, it has been reported that intermediate filaments are associated with mitochondria and sarcoplasmic reticulum.^{5,6)} They can influence mitochondria function by stabilizing their position at the areas of high energy demand or by stretching or contracting of the mitochondrial membrane.^{7,8)} Furthermore, they connected with the nucleus and the Z discs of the perinuclear myofibrils.⁹⁾ Clinically, there were some reports that a decrease of desmin was associated with certain diseases such as cardiomyopathy.^{8,29)} The cardiac myocytes in desmin knockout mice (Des^{-/-}) also defected the cytoarchitecture and mitochondria function, and showed swollen cytoplasm.²⁴⁾

Although auricular myocytes possessed a lot of atrial specific granules (ANP), there were few granules found in the BB. Thus, myocytes in the BB were different in ultrastructure from auricular myocytes.

Recently, the intercalated disks in the working cardiac myocytes were visualized three-dimensionally. They showed a stair-like profile, and consisted of steps (plicate segments) and risers (interplicate segments). The steps had a great number of microprojections. Many desmosomes, which meant the strength against mechanical stress, existed on the plicate segments. In the present SEM and TEM observations, the intercalated disks in the BB showed an ambiguous zigzag, and microprojections on the steps were larger in size and smaller in number than those in auricular myocytes, but gap junctions were very large.

Recently some diseases have been studied in association with these components. It was reported that sterile pericarditis decreased Cx40 and Cx43, especially at the endocardium, and induced atrial fibrillation.²⁷⁾ Furthermore, other reported that Cx43 could serve to prevent post-infarct arrhythmia.²⁸⁾

Conclusion

Based on present morphological findings, the BB was fundamentally different from atrial working myocytes, and showed properties of the cardiac conduction system.

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